JTCM 中医杂志

Online Submissions: http://www.journaltcm.com
info@journaltcm.com

J Tradit Chin Med 2012 September 15; 32(3): 1-2
ISSN 0255-2922

BASIC INVESTIGATION

Gas chromatography-mass spectrometry analysis on compounds in volatile oils extracted from Yuan Zhi (Radix Polygalae) and Shi Chang Pu (Acorus Tatarinowii) by supercritical CO_2

Yingli Wang, Liping Chang, Xiaoguang Zhao, Xianbin Meng, Yaming Liu

Xiaoguang Zhao, Department of Chinese Pharmacology, Shanxi University of Chinese Medicine, Shanxi, Taiyuan 030024, China
Yingli Wang, Department of Chinese Pharmacology, Shanxi University of Chinese Medicine, Shanxi, Taiyuan 030024, China; Key Laboratory of Coal Science and Technology, Taiyuan University of Technology, Ministry of Education and Shanxi Province, Shanxi, Taiyuan 030024, China
Liping Chang, Xianbin Meng, Yaming Liu, Key Laboratory of Coal Science and Technology, Taiyuan University of Technology, Ministry of Education and Shanxi Province, Shanxi, Taiyuan 030024, China
Supported by Shanxi Science and Technology Tackling Fund (No.20100311090), and Shanxi Provincial Health Department Science and Technology Tackling Fund (No.2008034)
Correspondence to: Prof. Yaming Liu, Key Laboratory of Coal Science and Technology, Taiyuan University of Technology, Ministry of Education and Shanxi Province, Shanxi, Taiyuan 030024, China. liuyaming66@yahoo.com.cn
Telephone: +86-351-2272163
Accepted: March 06, 2012

Abstract

OBJECTIVE: To analyze the constituents of volatile oils extracted from Yuan Zhi (Radix Polygalae), Shi Chang Pu (Acorus Tatarinowii), and a mixture of the two herbs.

METHODS: The volatile oils were extracted using supercritical fluid extraction (SFE) with CO_2, and the constituents of the volatile oil extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). The relative content of each component was calculated using peak area normalization.

RESULTS: The optimized SFE conditions were 45 MPa at 35°C for 2 h. Twenty-four compounds were identified in the extract from the Yuan Zhi (Radix Polygalae) and Shi Chang Pu (Acorus Tatarinowii) mixture, and six of these had relative contents >1. These compounds were 1,2-dimethoxy-4-(2-propenyl)-benzene; 1,2,3-trimethoxy-5-(2-propenyl)-benzene; β-asarone; (Z,Z) 9,12-octadecadienoic acid; (Z) 6-octadecenoic acid; and ethyl oleate. Combination of the herbs increased the number of pharmacologically active substances in the extract and decreased the number of compounds with one benzene ring compared with the extracts from the individual herbs.

CONCLUSION: These results indicate there is a synergistic relationship among the compounds in these herbs.

Key words: Gas chromatography-mass spectrometry; Radix polygalae; Acorus tatarinowii; Chromatography, Supercritical fluid

INTRODUCTION

The herbs Yuan Zhi (Radix Polygalae) from the dried root of Polygala tenuifolia Willd. and Shi Chang Pu (Acorus Tatarinowii) from the dried rhizome of Acorus gramineus, are used in traditional Chinese medicine. Their use was first recorded in Sheng Ji Zong Lu.[1] The main components of volatile oils extracted from these herbs are linoleic acid, oleic acid, palmitic acid, and asarone,[2,3] and these compounds are pharmacologically ac-

tive.[4-7] Recent research has shown that when these herbs are used together their pharmacological functions are enhanced compared with when they are used individually.[7]

Volatile oils are usually obtained from these herbs by steam extraction, but this process is time consuming, has a low yield and the operation temperature is high. The high temperature can lead to oxidation of the active ingredients and extraction of only a small number of chemicals. By comparison, supercritical fluid extraction (SFE) is conducted at a lower temperature, which preserves heat-sensitive volatile chemicals, and the extract contains minimal solvent residues. Volatile oil from SFE method contains a wider range of chemicals than that from steam extraction.

In this study, SFE was used to extract volatile oils from Yuan Zhi and Shi Chang Pu to minimize oxidation of the volatile components. GC-MS was used to identify some of the compounds in the volatile oils. These results could be used as a reference for further pharmacological research and investigations of compound activity.

METHODS

Material and instruments

P. tenuifolia Willd. (batch No. 090109) and A. gramineus (batch No. 100319) were harvested from Shanxi and Henan provinces (China) respectively. The herbal medicines were produced by Bozhou Chinese Medicine Limited Company(Bozhou, China). Both samples were identified for this experiment by Prof. Xiang-Ping Pei, an expert from Shanxi University of Chinese Medicine (Taiyuan, China). Chloroform (chromatography grade, Beijing Chemical Plant, Beijing, China) was used for extraction. A SFE unit (Applied Separations, Allentown, PA) and an Agilent 6890N-5973 inert GC/MS (Agilent Technologies, Santa Clara, CA) were used for extraction and analysis of the volatile oils, respectively.

SFE extraction

Yuan Zhi (and Shi Chang Pu were mixed together (1∶1 mass ratio), and 24 g of this mixture was extracted in a 30 mL reactor. The effects of temperature, pressure, and extraction time on the volatile oil extraction yield were investigated.

After the conditions were optimized, the extraction process was applied to 24 g the mixture (1∶1 mass ratio), 24 g of Yuan Zhi, and 21.7 g of Shi Chang Pu. The three volatile oils obtained were labeled POACVO for the mixture, POVO for Yuan Zhi, and ACVO for Shi Chang Pu.

GC-MS operating conditions

The GC separation was performed on a 19091S-433 HP-5MS elastic quartz capillary column (30.0 m × 0.25 mm I.D., film thickness 0.25 μm, Agilent Technologies). The column temperature was held at 55°C for 1 min, increased to 240°C at 3°C/min, and held at this temperature for 17 min. The carrier gas was nitrogen which flow rate was 1.0 mL/min. A 1 μL sample was injected at of 230°C. The pre-column pressure was 14.5 MPa, and the inlet/outlet ratio was 20∶1.

The MS was operated with an electron ionization power of 70 eV, ion source temperature of 230°C, and scan range of 35-500 amu.

Testing method

An aliquot (10 μL) of the volatile oil extracted from the mixture of the two herbs was diluted to 1.0 mL with chloroform, and 1.0 μL of the diluted sample was analyzed by GC-MS. NIST 0.2L was used to process the spectra. The volatile oils extracted form the individual herbs were analyzed by the same procedure.

RESULTS

Effect of pressure

Pressure and temperature are critical variables in supercritical CO_2 extraction as they determine the density and solvating power of supercritical CO_2.[8] The effect of pressure (20, 30, 40, 45, and 50 MPa) on the oil yield was investigated at 35° C with an extraction time of 30 min (Figure 1).

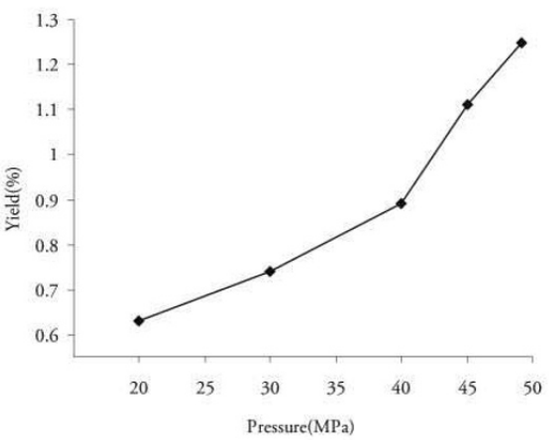

Figure 1 Effect of pressures on the extraction at 35°C for 30 min

As the increased pressure, the volatile oil yield also increased. At a constant temperature, when the pressure increases, the density of supercritical CO_2 increases, the solvating power increases, and the efficiency of mass transfer between the fluid and volatile oil is enhanced.[9]

As the pressure increased from 40 MPa to 45 MPa, the oil yield increased from 0.90 % to 1.11%, and from 45 MPa to 50 MPa, the oil yield improved from 1.11% to 1.26%. Taking the limit for the operation pressure into account (<68 MPa) and energy consumption, 45 MPa was selected as the optimal extraction pressure.

Effect of temperature

The effect of temperature on the extraction was investi-

gated from 30-45°C at 45 MPa for 30 min (Figure 2). The optimum temperature for maximizing the volatile oil yield was 35°C. This was determined by the effect of temperature on the supercritical fluid.[10] The temperature affects the solvating power through two competing factors, solute sublimation, and supercritical fluid solvent density. When the temperature increases, the solute sublimation pressure increases, and the solvent density decreases. These temperature-dependent factors affect the extraction yield in opposing ways.[11] From 30-35°C, the solubility of the solute increased despite the decrease in the supercritical fluid solvent density, and this enhanced the extraction process. From 35-45° C, the solute solubility decreased, and the volatile oil extraction yield decreased. Therefore, in consideration of these two effects, 35° C was selected as the optimum temperature.

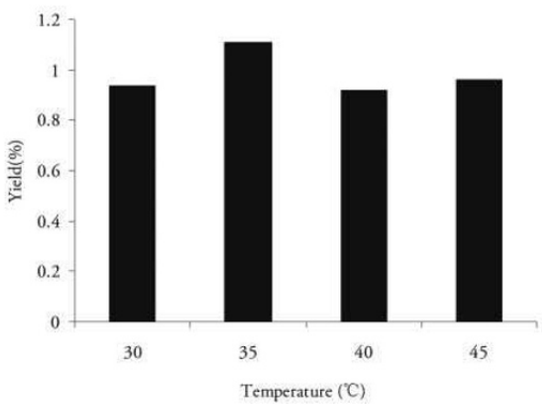

Figure 2 Effect of temperature on the extraction yield at 45 MPa for 30 min

Effect of extraction time

The effect of the extraction time (20-120 min) on the yield was examined at 45 MPa and 35°C (Figure 3).

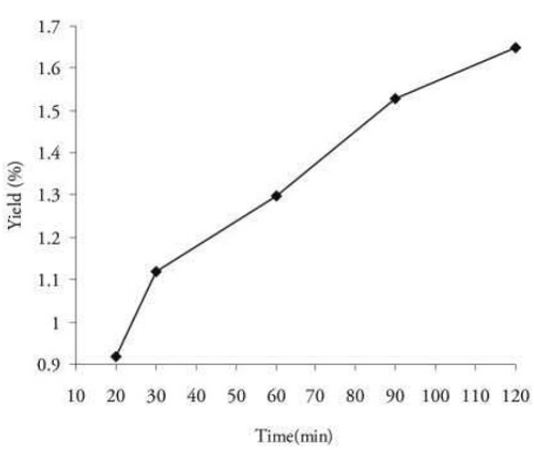

Figure 3 Effect of the extraction time on the extraction yield at 45 MPa and 35°C

As the extraction time was increased from 20 min to 120 min, the yield was increased gradually from 0.92% to 1.63%. A longer supercritical CO_2 extraction time would change the volatile oil composition and increase energy consumption. In consideration of SFE unit lifespan, 120 min was selected as the optimum extraction time.

In summary, the optimized conditions for the SFE were 45 MPa, 35°C, and 120 min. Under these conditions, the volatile oil yields for SFE of Yuan Zhi, Shi Chang Pu, and the 1∶1 mixture of the two herbs were higher than those obtained by steam extraction technology by 0.67%, 2.38%, and 1.63%, respectively.[3] The oil obtained was clear and green (POVO), yellow-brown (ACVO), or brown-green (POACVO).

GC/MS analysis of the volatile oils

Total ion current chromatograms for the three volatile oils are show in Figure 4 (POACVO), Figure 5 (POVO), and Figure 6 (ACVO). Compounds identified in the GC-MS results that had relative contents >0.1% are listed in Table 1.

In POVO, 30 peaks were recorded and 18 of the compounds were identified. Among these compounds, the following eight had relative contents >1%∶1,2,3-trimethoxy-5-(2-propenyl)-benzene; α-asarone; n-hexadecanoic acid; 8-octadecenoic acid, methyl ester; (E)-9-octadecenoic acid; (Z)-6-octadecenoic acid; 9,12-octadecadienoic acid, ethyl ester; and ethyl oleate. 6-Octadecenoic acid had the highest relative content (50.31%). The relative content of 9,12-octadecadienoic acid, ethyl ester, which was the major bioactive compound detected, was 3.54%.

In ACVO, 57 peaks were recorded and 22 of the compounds were identified. Among these compounds, the following five had relative contents >1%∶ 1,2-dimethoxy-4-(2-propenyl)-benzene; 1,2,3-trimethoxy-5-(2-propenyl)-benzene; β-asarone; α-cadinol; and (Z, Z)-9,12-octadecadienoic acid. The major bioactive compound, β-asarone, was also the most abundant (70.93 %).

For the 48 peaks detected in POACVO, 24 compounds were identified. The following six compounds had relative contents >1%∶ 1,2-dimethoxy-4-(2-propnyl)-benzene; 1,2,3-trimethoxy-5-(2-propenyl)-benzene; β-asarone; (Z,Z)-9,12-octadecadienoic acid; (Z)-6-octadecenoic acid; and ethyl ester. Four of these compounds were also abundant in ACVO. β-asarone was identified as the most abundant (63.33%) compound in POACVO.

Two types of compounds were detected in POVO, both with one benzene ring in their molecular structure, and the total relative content of these compounds was 0.77%. In ACVO, four types of compounds were identified, and the total relative content was 1.29%. In POACVO, only one type of compound was identified, and the total relative content was 0.16%. These results indicate combination of the two herbs decreased both the number of a single benzene ring compounds in the extract and their relative contents. In addition, four compounds were detected in POACVO that were not detected in the extracts from the individual herbs. They were D-camphor, a-selinene, a-muurolene, and (E)-9-octadecenoic acid, methyl ester. Among these compounds, (E)-9-octadecenoic acid, methyl ester is

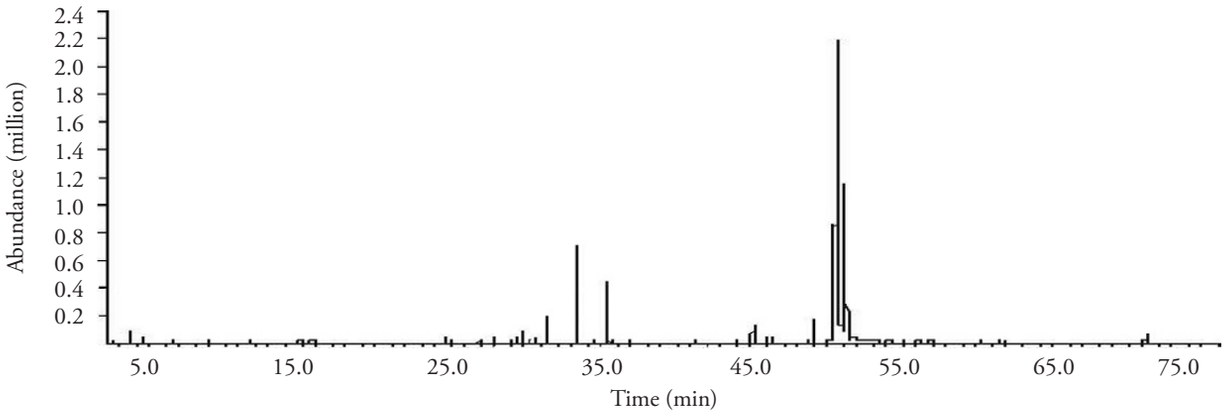

Figure 4 Total ion current chromatogram of the SFE-CO_2 extract of Yuan Zhi

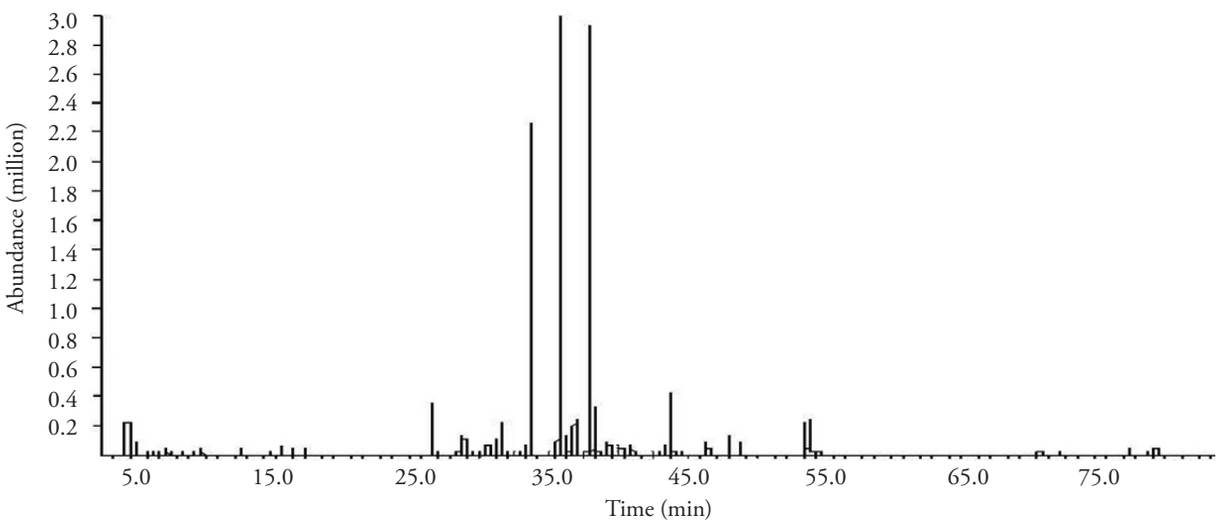

Figure 5 Total ion current chromatogram of the SFE-CO_2 extract of Shi Chang Pu

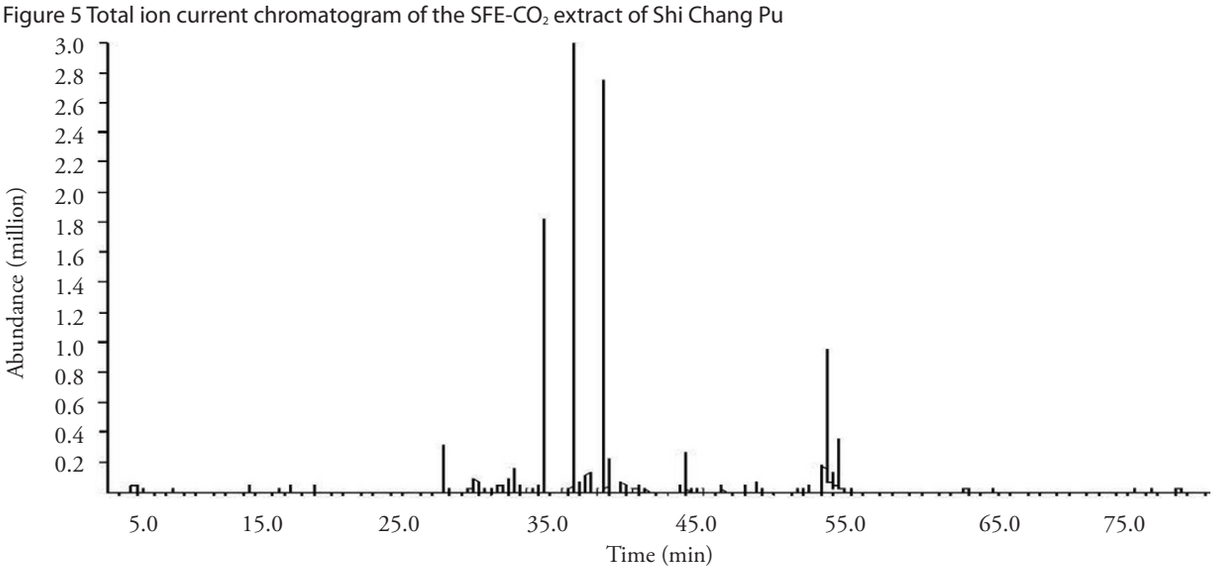

Figure 6 Total ion current chromatogram of the SFE-CO_2 extract of a mixture of Yuan Zhi and Shi Chang Pu

new, and could be produced by combination of the two herbs.

DISCUSSION

CO_2 SFE uses a lower operation temperature than traditional steam extraction. This effectively reduces decomposition of heat-sensitive ingredients during extraction and separation.[2] After extraction at 45 MPa and 35°C for 120 min, the volatile oil yields from SFE were higher than those obtained by steam extraction.

This study demonstrates for the first time that combined use of Yuan Zhi and Shi Chang Pu can decrease the content, and therefore, activity of compounds containing a single benzene ring in the extract.

POACVO contains the following five pharmacologically active ingredients: 1, 2, 3-trimethoxy-5-(2-propenyl)-benzene; β-asarone; (Z,Z)-9,12-octadecadienoic acid; n-hexadecanoic acid; and 9,12-octadecadienoic acid, ethyl ester.

Table 1 Compounds and their relative contents in the volatile oils extracted by SPE with CO_2 from the herbs Yuan Zhi (POVO), Shi Chang Pu (ACVO) and their mixture (POACVO)

Name / Molecular Formula	Area(%)/R.T. (min)		
	POVO	ACVO	POACVO
Ethylbenzene / C_8H_{10}	0.16/4.64	0.24/4.64	
p-Xylene / C_8H_{10}	0.61/4.81	0.62/4.82	0.16/4.82
m-Xylene / C_8H_{10}		0.24/5.38	
3-Ethyltoluene / C_9H_{12}		0.19/7.35	
Linalool / $C_{10}H_{18}O$		0.17/12.59	0.17/12.60
D-CAMPHOR/ $C_{10}H_{16}O$			0.11/14.45
L(-)-Borneol / $C_{10}H_{18}O$		0.19/15.35	0.17/15.36
Naphthalene ball /$C_{10}H_8$		0.19/16.01	
Anisole, p-allyl- / $C_{10}H_{12}O$	0.11/16.81	0.10/16.80	0.14/16.81
Benzene, 1,2-dimethoxy-4-(2-propen yl)-/$C_{11}H_{14}O_2$	0.30/25.71	1.31/25.71	1.43/25.72
alpha-Selinene/ $C_{15}H_{24}$			0.12/28.93
(-)-alpha-Gurjunene / $C_{15}H_{24}$		0.25/29.19	0.27/29.19
Benzene, 1,2-dimethoxy-4-(1-propenyl)-/ $C_{11}H_{14}O_2$		0.39/29.47	0.71/29.47
alpha-Muurolene/ $C_{15}H_{24}$			0.10/29.53
gamma-Cadinene/ $C_{15}H_{24}$		0.43/30.06	0.45/30.07
1H-Cyclopropa[a]naphthalene, 1a,2, 3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alp ha.,7a.alpha.,7b.alpha.)]-/ $C_{15}H_{24}$	0.11/30.06		
delta-Cadinene/ $C_{15}H_{24}$		0.91/30.45	0.90/30.46
beta -Cadina / $C_{15}H_{24}$	0.29/30.46		
Elixene/ $C_{15}H_{24}$	0.22/31.70		
Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-/ $C_{12}H_{16}O_3$	1.35/32.50	9.78/32.54	9.96/32.53
Asarone / $C_{12}H_{16}O_3$	8.75/34.31		
Benzene, 1,2,4-trimethoxy-5-(1-pro penyl)-, (Z)-/ $C_{12}H_{16}O_3$		70.93/34.48	63.33/34.43
.tau.-Muurolol/ $C_{15}H_{26}O$		0.88/34.98	0.50/34.96
α-Copaene / $C_{15}H_{24}$		0.16/35.14	
α-cadinol / $C_{15}H_{26}O$		1.09/35.45	0.73/35.44
Propiophenone, 3',4'-dimethoxy-/ $C_{11}H_{14}O_3$		0.14/35.62	0.10/35.61
2,4,5-trimethoxybenzaldehyde / $C_{10}H_{12}O_4$		0.27/37.82	0.30/37.82
n-Hexadecanoic acid/ $C_{16}H_{32}O_2$	1.48/46.05	0.67/46.03	0.34/46.00
Hexadecanoic acid, ethyl ester/$_{18}H_{36}O_2$	0.36/47.05		
9,12-Octadecadienoic acid, methylester / $C_{19}H_{34}O_2$	0.27/50.07		
8-Octadecenoic acid, methyl ester / $C_{19}H_{36}O_2$	1.32/50.27		
9-Octadecenoic acid, methyl ester, (E)-/ $C_{19}H_{36}O_2$			0.25/50.27
9,12-Octadecadienoic acid (Z,Z)-/ $C_{18}H_{32}O_2$		2.67/51.28	1.80/51.34
9-Octadecenoic acid, (E)-/ $C_{18}H_{34}O_2$	17.89/51.54		
6-Octadecenoic acid, (Z)-/ $C_{18}H_{34}O_2$	50.31/51.79		10.29/51.57
9,12-Octadecadienoic acid, ethyl ester/ $C_{20}H_{36}O_2$	3.54/52.11		0.57/52.09
Ethyl Oleate / $C_{20}H_{38}O_2$	9.85/52.29		1.86/52.27
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-, (all-E)-/ $C_{30}H_{50}$	0.85/72.18		

Compared withSFE of the individual herbs, SFE of a mixture of the two herbs increases the number of bioactive ingredients extracted compared with extraction of the individual herbs, changes the relative contents of the compounds in the extract, reduces extraction of compounds containing a single benzene ring. These quantitative and qualitative changes in the bioactive ingredients will result in the herbs having different effects if used alone or in combination. The results indicate the herbs are reasonably compatible for co-administration, and this provides strong support for their combination in clinical practice.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Prof. Yan Ni for technical advice.

REFERENCES

1 **Lu JS** . Herb-pairs of Shi Jin Mo. 3rd ed. Bei Jing: People's Military Medical Press; 2007: 265-266.

2 **Jin JZ**, Ha CY. Chemical constituents of volatile oil from Rhizomes of Acorus Gramineus by supercritical fluid extraction. Zhong Cao Yao 2007; 38(8): 1159-1160.

3 **Fang MF**, Wang R, Zhang WJ, Hu SS, Li YF, Zheng XJ. GC/MS analysis volatile oil of Polygala Tenuifolia Willd. - Acorus Tatarinowii Schott herb pairs. Zhong Cheng Yao 2010; 32(2): 311-314.

4 **Li L**, Zhou YY, Shi C, Wei G, Lin SF, Fang YQ. Comparative investigation on the drug effect of β-asarone, α-asarone and volatile oil of Acorus tatarinowii Schott. on bronchial asthma. Shi Zhen Guo Yi Yu Guo Yao 2006; 17(11): 2137-2138.

5 **Wu QD**, Wu QH, Wang QW, Chen YZ. Study on anti-thrombosis effect of volatile oil of Acorus Tatarinowii Schott. And β-asarone. Zhong Yao Xin Yao Yu Lin Chuang Yao Li 2008; 19(1): 29-31.

6 **Li Z**, Chen Z. Oleic acid in the metabolic syndrome.Ya Tai Chuan Tong Yi Yao 2009; 5(2): 18-20.

7 **Zheng LP**, Yan YX, Lin JM, Chen XF. Study of Polygala Tenuifolia Willd. - Acorus Tatarinowii Schott decoction mixture on the aging effect in mice led by D-galactose. Fujian Zhong Yi Yao 2002; 33(4): 35-36.

8 **Tang Sk**, Li SF, Ye Ch, Tian SJ. Extraction of grape seed oil from grape seed with supercritical CO2. Gao Xiao Hua Xue Gong Cheng Xue Bao 2004; 18(1): 23-27.

9 **Lun CM**, Zhao XL, Liu ZQ, Xing JP. Isolation and extraction of total flavonoids from Epimedium Koreanum Nakai by supercritical fluid extraction. Chem Res Chinese U 2004; 20(6): 707-710.

10 **Lun CM**, Zhao JM, Li HM, Song FR. Supercritical fluid extraction of total flavonoids from leaves of Acanthopanax Senticosus harms. Chem Res Chinese U2007; 23(2): 233-236.

11 **Huang Z**, Yang MJ, Liu SF, Ma Q. Supercritical carbon dioxide extraction of Baizhu: experiments and modeling. J of Supercritical Fluids 2011; (58): 31-39.